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Biopotentials of Mangroves Collected from the Southwest Coast of India

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Abstarct: Southwest coast of India boasts remarkable biodiversity and presents a pristine seascape. In the present study, three mangrove species (*Avicennia marina*, *Bruguiera cylindrical* and *Acanthus ilicifolius*) collected form the coast was extracted in methanol and tested for different range of biological activities including antimicrobial activity against five species of type cultures (Microbial Type Culture Collection) of fish/shrimp *Vibrio* pathogens, brine shrimp cytotoxic, antifouling and ichthyotoxic activities. The overall activity profile showed that, *A. marina* exhibited more biopotency than *B. cylindrica* and *A. ilicifolius.* The highly active mangrove, *A. marina* was evaluated further to analyse the active compounds using gas chromatography. The analysis revealed a mixture of fatty acids such as alpha linolenic acid (30%), palmetic acid (21%), stearic acid (14%), lauric acid (9%), myristic acid (5%), oliec acid (5%) which might have functional role in bioactivity and can be used for the development of biodegradable antifoulants, pisicides and biopharmaceuticals.

Key words: Mangrove extract · Vibriocidal activity · Brine shrimp cytotoxicity · Antifouling activity

INTRODUCTION

Mangroves are intertidal productive forested wetland constrained to the tropical and subtropical estuarine zones, serves as a nursery, feeding and spawning ground for commercial finfishes and shell fishes [1]. Habitat of mangrove plants is commonly known as mangrove swamps, tidal forests, tidal swamp forests or mangals [2]. These vascular halophytic plants constitute a vital component of marine flora and have significant ecological and socio-economic values. For centuries, mangroves have been traditionally used for food (fruits and nectar) feed and medicinal purposes in different parts of the world. They are well known to produce natural metabolites with diverse biological activities such as antibacterial [3] antiviral activity [4], antidiarrhoeal activity [5] antifeedant activity [6] insecticidal activity [7] and cytotoxic activity [8] However, during the last decade screening of mangroves for bioactive active compounds, has received high interest as a potential bioresource for novel drug leads. Until now, more than 200 bioactive metabolites have been isolated from true mangroves of tropical and subtropical populations [6]. According to their chemical structure, most of the isolated compounds belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics which having a wide range of therapeutic possibilities [9].

Approximately 55 species of mangroves from 22 genera were distributed in Indian ocean region [10]. The first report on regarding the chemistry of Indian mangroves was reported by Rao and Bose [11]. Recent research evidenced that Indian mangroves contained antibacterial [12], antiviral [13], mosquito larvicidal [14], antifungal [15] and antioxidant activity [16].

The biological activity of seaweeds from the southwest cost of India (Kollam coast) is already reported [17, 18]. Hitherto, mangroves of the southwest coast (Kollam coast) have not yet been studied for their biological activity. In light of this, the present study was initiated to investigate the biopotentials of mangroves from the southwest coast of India (Kollam coast) against a different range of activity including shrimp vibriocidal, brineshrimp cytotoxic, antifouling and ichthyotoxic activity.

MATERIALS AND METHODS

Collection and Extraction of Mangrove Bioactives: Three species of mangroves viz., *Avicennia marina* (Forsk.) (Avicenniaceae), *Bruguiera cylindrica* (Rhizophoraceae) and *Acanthus ilicifolius* (Acanthaceae) were collected and identified from mangrove forest of Ayiramthengu located in Kollam (08º54'N and 76º38'E) area (southwest coast of India) (Fig. 1) at various dates

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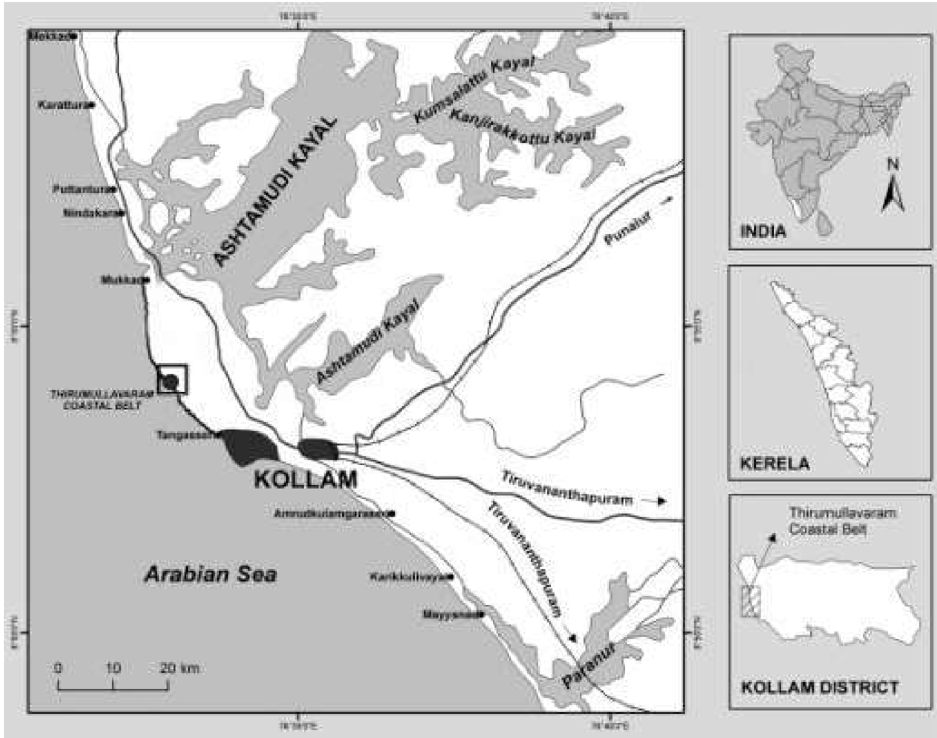

Fig. 1: Map showing the study area, (Kollam coast) southwest coast of India

from April 2008 to April 2009. Prior to the extraction the leaves of respective species were cleaned, shade dried in order to prevent photolysis and thermal degradation, chopped into small pieces and ground coarsely in a mechanical grinder.

Extraction of Bioactives: For extraction of crude bioactives, 100 g of powdered mangrove material was refluxed three times in a 1 liter capacity round bottom flask in a water bath at 65ºC for about 6 h using methanol. The extracts were filtered and concentrated to recover the excess solvents in another distillation system. The concentrated extract (about 100ml) was again filtered through a Whatman no. 1 filter paper fitted with a Buchner funnel using suction pressure. Finally, it was reduced to thick oily natured crude extract in a rotary vacuum evaporator (Yamato) at 40°C, collected in air–tight plastic vials and stored in the refrigerator for further activity studies

Bioassays: Antimicrobial activity was carried out as described by Selvin and Lipton [19] against five species of type cultures (Microbial Type Culture Collection, MTCC) of fish/shrimp *Vibrio* pathogens such as *V. harveyi* (MTCC 3438), *V. alginolyticus* (MTCC 4439), *V. vulnificus* (MTCC 1145), *V. parahaemolyticus* (MTCC 451) and *V. alcaligenes* (MTCC 4442). The cytotoxic activity of mangrove extracts was tested against freshly hatched free-swimming nauplii of *Artemia salina* (Linnaeus) (*Artemia salina*, Sanders Great Salt Lake, Brine Shrimp Company L.C., U.S.A.). The assay system was prepared with 2 mL of filtered seawater containing chosen concentration of extract in cavity blocks (embryo cup) and 20 nauplii each was transferred in experimental, vehicle control and negative control wells. Invariably the concentration of the experimental systems was determined on the basis of exploratory experiments. The percentage of mortality was determined by comparing the mean surviving larvae of the test and control tubes. The LD_{50} value was determined using probit scale [20]. Fingerlings (1.5-2.0 cm) of marine acclimated *Oreochromis mossambicus* were used for evaluating the ichthyotoxic potential [19]. Antifouling activity was evaluated against common rock fouler, *Patella vulgata* using 'mollusc foot adherence bioassay' [19]. All the experiments were performed in the present study repeated six times to validate the findings statistically.

Gas Chromatographic Analysis of Active Mangrove, *A. Marina:* The methanolic extracts of *A. marina* (10 gm) laoded on a silica gel (60-120 mesh) (E. Merck) column packed with hexane and eluted with hexane and chloroform (9:1 to 1:9 and 100% chroloroform) followed by ethyl acetate and methanol (9:1 to 1:9 and 100% methanol) to yield fourteen fractions. Individual fractions were collected and tested for bioactivity (data not shown). The fraction that was eluted using chloroform and ethyl acetate (2:8) exhibiting activity was subjected to Gas chromatography. A Gas Chromatgraph (Shimadzu 2014) equipped with Flame Ionization Detector {FID} and column DB-225 (0.25×15mm) was used for the analysis.

RESULTS AND DISCUSSION

The plant material was subjected to an extraction process, with methanol. The yields were 3.8% for the *A. marina* extract, 3.2% for *B. cylindrical* extract and 4.2% for the *A. ilicifolius* extract.

Antibacterial Activity: The *invitro* antibacterial activity revealed that the methanolic extract of mangroves had remarkable vibriocidal activity. Among the three species tested, *A. marina* exhibited wide spectrum of activity which suppress the growth of all tested vibrios, produced a mean zones of inhibition of more than 14 mm (Fig. 2). *A. ilicifolius* was found to be active against three species of vibrios such as *V. alcaligenes* (8mm), *V. vulnificus* (9 mm) and *V. alginolyticus* (10 mm) while the extract of *B. cylindrica* had the lowest activity which inhibit the growth of only two bacteria, *V. alcaligenes* (7 mm) and *V. alginolyticus* (10 mm). The difference between the antimicrobial activities of mangroves could be due to the quantity of antimicrobial substances present in each form. The sensitivity of *V. alcaligenes* to all of the mangrove extracts could be attributed due the presence of common bioactive compounds that had inhibitory effects on the microorganism.

In comparison to our study, antibacterial activity of mangroves against fish pathogens had already been studied by many authors. Abou-Elela *et al*. [21] reported the root extracts of *A. marina* had vibriocidal activity against *V. fluvialis* and *V. vulnificus*. Choudhury *et al*. [22] noted that methanolic extract of *A. cucullata* had growth inhibition against the fish pathogen, *V. alginolyticus*. Similarly, Mishra and Sree [23] reported the chloroform leaf extract of *Finlaysonia obovata* showed strong antibacterial activity against fish pathogens.

Brine Shrimp Assay: The brine shrimp assay is considered as a reliable indicator for the preliminary assessment of toxicity [24] and it can be extrapolated for cell line toxicity and anti-tumour activity. This assay is

Table 1: *Artemia* cytotoxicity profile of mangrove extracts

Mangrove extracts	Concentrations (µg/ml)	Mortality (%)
A. marina	200	13.8±2.6
	400	66.3±1.8
	600	100±0.0
B. cylindrica	200	9.5±3.2
	400	43.8±4.7
	600	93.6± 2.5
A. ilicifolius	200	8.5±1.6
	400	36.9±3.3
	600	87.3±4.2

Mean ± SD n = 6

Table 2: Ichthyotoxicity profile of mangrove extracts to *Oreochromis mossambicus* fingerlings

Species	Concentrations (µg/ml)	Mortality (%)	Time of death
A. marina	100	20.5±2.3	12
	150	58.4±2.6	8
	200	100±0.0	4
B. cylindrica	150	10±3.7	12
	200	60.5±4.6	8
	250	100 ±0.0	4
A. ilicifolius	200	30±5.5	12
	250	55.3±3.9	8
	300	100 ±0.0	4

Mean ± SD n = 6

Table 3: Antifouling profile of mangrove extracts

Species	Concentrations (mg/ml)	Fouling rate (%)	Regaining rate (%)
A. marina	2	70±2.5	100
	4.7	30±4.2	100
	6.3	0	80
	8.2	0	30
B. cylindrica	11.5	20±1.2	50
A. ilicifolius	14.8	10±2.5	20

Mean ± SD n = 6

Table 4: Fatty acid composition of the active fraction of *A. marina*

Peak	Retention time (min)	Area	Area%	Name
1	2.134	2527	8.5693	Unidentified
2	2.431	1771	6.0059	Unidentified
3	2.821	2773	9.4013	Lauric acid
6	2.990	1489	5.0498	Myristic acid
7	3.736	6227	21.1162	Palmitic acid
8	5.495	4175	14.1579	Stearic acid
9	5.657	1660	5.6292	Oliec acid
10	7.168	8868	30.0703	alpha linolenic acid

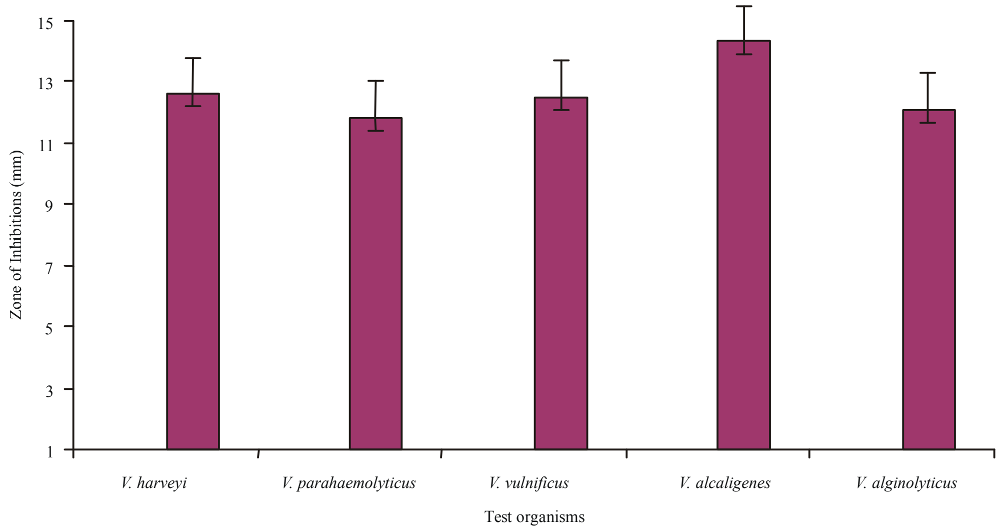

Fig. 2*:* Antibacterial activity of *A. marina*

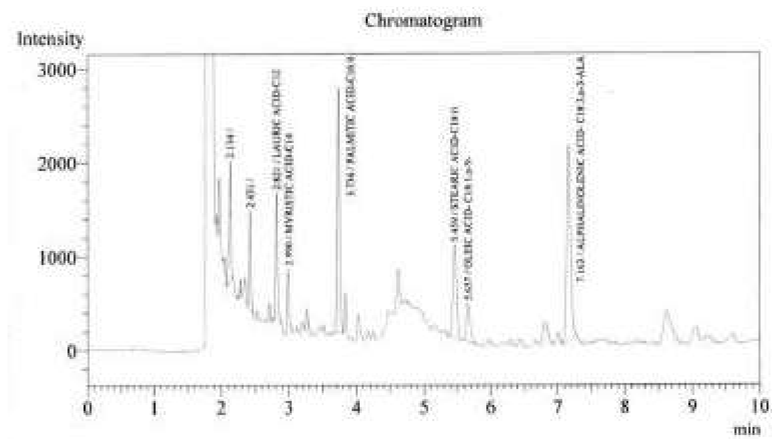

Fig. 3: GC profile of active column chromatography fraction of *A. marina*

widely employed in the screening process of botanical for the isolation of bioactive metabolites. In the present study, the extract of three mangrove species showed different mortality rate at different concentrations (Table 1). The mortality rate increased with the increase of concentration of each sample. The crude extracts of *A. marina* indicated the highest activity with LD_{50} value of 318 µg/ml. The extracts of *B. cylindrica* and *A. ilicifolius* exhibited weaker brine shrimp lethality with LD_{50} values of 410 and 475 µg/ml, respectively.

Haque *et al*., [25] reported the cytotoxic activity of *Xylocarpus mollucensis* against brine shrimp nauplii. There are numerous reports of mangrove metabolites with cytotoxic activity [26]. Secondary metabolites which have cytotoxic activity include, diterpenoids from genus of *Bruguiera* [27]; Naphthoquinones from genus of *Avicennia* [8]; the mansonones, extracted from the heartwood of *T. populnea* [28] and alkaloids of *Alstonia macrophylla* [26]. The results implies that mangroves of the southwest coast of India contain potential bioactive compounds, which could be utilized for the development of novel anticancer leads.

Ichthyotoxic Activity: According to Yoshida and Ito, [29] ichthyotoxic assay is a preliminary screening method used to search for novel natural products. Natural ichthyotoxic metabolites often have a diversity of other biological activities, such as insecticidal [30] and anti-tumor [31]. Literature point out that ichthyotoxic properties of mangrove plants from different parts of the world have been discovered long ago [32-34].

In the present study, the *in vitro* ichthyotoxic activity was considered as the ability of mangrove extract to slay the fishes in respective concentration. The results showed that the mangrove extracts produced toxicity at different concentration (Table 2). At a concentration of 200 µg/ml, *A. marina* showed 100% mortality in tilapia after 4 h. The extract of *B. cylindrica* and *A. ilicifolius* exhibited 100% mortality at 250 and 300 µg/ml respectively. The mode of action of mangrove may be due to the inhibition of nervous system of fish. It was found that the mangrove extracts impart more or less same sort of behavioral changes in fishes [17]. Bandaranayake, [26] reviewed that saponins are the main factor responsible for the piscicidal activity. In recent years, a lot of work has been done on ichthyotoxic properties of mangroves, e.g. Benzoquinones embelin and 5-O-methyl embelin, from *Aegiceras corniculatum* [26], sapintoxin A from the poisonous plant *Sapium indicum* and Balanitin from *B. aegyptaica* [35] exhibited strong pisicidal activity. Early report envisages that Indian mangroves also had promising ichthyotoxic properties [36]. Based on the present findings, it could be inferred that mangrove extracts can be used for the management of weed and predatory fishes.

Antifouling Activity: Use of natural products to control fouling organisms has been studied by various people worldwide [37,38,19]. The extracts of mangroves were reported to have antifouling properties [39]. In the present study, bioactivity of mangrove extracts was based on the adherence (fouling) or shrinkage of the foot. Significant antifouling activity against *P. vulgata* was observed with methanol extract from *A. marina*, followed by extracts of *B. cylindrica* and *A. ilicifolius* (Table 3). The extract of *A. marina* exhibited complete inhibition of foot adherence/fouling at a concentration of 6 mg/ml. This concentration was considered as a safe dose, as 80% of the exposed *P. vulgata* were regained after the treatment period. However, there existed a range in mortality according to the concentration of extracts incorporated in the treatment. It was found that *A. marina* was relatively more toxic at 10 mg/ml, in which 100% mortality was occurred. Similar observations on other plant extracts have been reported [19]. These preliminary data suggest that the methanolic extract of *A. marina* may be used to develop environmental safe antifoulant to control fouling organisms.

Gas Chromatographic Analysis *of A. Marina*: The gas chromatography results of active column fraction revealed that the active principals were a mixture of fatty acids ranging from C-12 to C-20 (Fig.2 & Table 4). The active column fraction of *A. marina* showed six fatty acids such as alpha linolenic acid (30%), palmetic acid (21%), stearic acid (14%), lauric acid (9%), myristic acid (5%) and oliec acid (5%). Recent research reported that many fatty acids from mangroves posses antimicrobial property [40-43].

CONCLUSION

Mangroves from the southwest coast of India were studied for the first time for bioactivity. From the preliminary screening, we have identified mangrove plants with pronounced biological activities against limpets, brine shrimp, fishes and shrimp vibrios. Among the three species screened, the broadest activity was showed by *A. marina*, therefore this mangrove might be a potential source for developing ecologically significant bioactive compounds including biodegradable antifoulants, pisicides and biopharmaceuticals. Moreover the vibriocidal property of *A. marina* can be utilized for the development of leads for aquaculture drug development.

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REFERENCES

1. Sunil Kumar, R, 2000. A review on biodiversity studies of soil dwelling organisms in Indian mangroves. Zoo's print Journal, 15(3): 221-227.
2. Kathiresan, K. and B.L. Bingham, 2001. Biology of mangrove and mangrove ecosystems. Advances in Marine Biology, 40: 81-251.
3. Abeysinghe, P.D., R.P. Wanigatunge and R.N. Pathirana, 2006. Evaluation of antibacterial activity of different mangrove plant extracts. Ruhuna Journal of Science, 1: 104-112.
4. Zandi, K., M. Taherzadeh, S. Tajbakhsh, R. Yaghoubi, Z. Rastian and K. Sartavi, 2008. Antiviral activity of *Avicennia marina* leaf extract on HSV-1 and Vaccine strain of polio virus in vero cells, International Journal of Infectious Diseases, 12(1): 298.

5. Rouf, R., S.J. Uddin, J.A. Shilpi and M. Alamgir, 2007. Assessment of antidiarrhoeal activity of the methanol extract of *Xylocarpus granatum* bark in mice model. Journal of Ethnopharmacology, 109: 539.
6. Wu, J., Q. Xiao, J. Xu, M.Y. Li, J.Y. Pana and M. Yang, 2008 Natural products from true mangrove flora: source, chemistry and bioactivities, Natural Product Report, 25: 955-981.
7. Calderon, J.S., C.L. Cespedes, R. Rosas, G.G. Federico, J. R. Salazar, L. Laura, A. Eduardo, K. Isao, 2001. Acetylcholinesterase and insect growth inhibitory activities of *Gutierrezia microcephala* on fall armyworm *Spodoptera frugiperda* J. E. Smith Zeitschrift fuer Naturforschung, Journal of Biosciences, 56: 382-394.
8. Han, L., X.S. Huang, I. Sattler, H.Z. Fu, S. Grabley and W.H.J. Lin, 2007. Two new constituents from mangrove *Bruguiera gymnorrhiza*. Journal of Asian Natural Product Research, 9: 327.
9. Bandaranayake, W.M., 1998. Traditional and medicinal uses of mangroves. Mangroves and Salt Marshes, 2: 133-148.
10. Kathiresan, K. and N. Rajendran, 2005. Mangrove ecosystems of Indian Ocean. Indian journal of marine sciences, 34(1): 104-113.
11. Rao, K.V. and P.K. Bose, 1959. A Genin- and isorhamnetin from the bark of *Aegiceras majus*. Journal of the Indian Chemical Society, 36: 358-60.
12. Chandrasekaran, M., K. Kannathasan, V. Venkatesalu and K. Prabhakar, 2009. Antibacterial activity of some salt marsh halophytes and mangrove plants against methicillin resistant Staphylococcus aureus World Journal Microbiology and Biotechnology, 25: 155-160.
13. Premanathan, M., R. Arakaki, H. Izumi, K. Kathiresan, M. Nakano and N. Yamamoto, 1999. Antiviral properties of a mangrove plant, *Rhizophora apiculata* blume against human immunodeficiency virus. Antiviral Research, 44: 113-122.
14. Thangam, T.S. and K. Kathiresan, 1989. Larvicidal effect of marine plant extracts on mosquito *Culex tritaeniorhynchus*. Journal of the Marine Biological Association of India, 31: 306-307.
15. Bose, S. and A. Bose, 2008. Antimicrobial activity of *Acanthus ilicifolius* (L.). Indian Journal of Pharmacy Science, 70: 821-3.
16. Babu, B.H., B.S. Shylesh, J. Padikkala, 2001. Antioxidant and hepatoprotective effect of *Acanthus ilicifolius*. Fitoterapia, 72: 272-277.
17. Manilal, A., S. Sujith, J. Selvin, G.S. Kiran, C. Shakir, R. Gandhimathi and M.V.N. Panikkar, 2009. Biopotentials Of Seaweeds Collected From Southwest Coast Of India. Journal of Marine Science and Technology, 17(1): 67-73.
18. Shanmughapriya, S., A. Manilal, S. Sujith, J. Selvin, G.S. Kiran and K. Natarajaseenivasan, 2008. Antimicrobial activity of seaweeds extracts against multiresistant pathogens. Annals of Microbiology, 58(3): 535-541.
19. Selvin, J. and A.P. Lipton, 2004. Biopotentials of *Ulva fasciata* and *Hypnea musiformis* collected from the peninsular coast of India. Journal of Marine Science and Technology, 12: 1-6.
20. Wardlaw, A.C., 1985. Practical statistics for experimental biologists, John Wiley and Sons, Chichester.
21. Abou-Elela, G.M., N.A. El-Sersy, M.A. El-Shenawy, H. Abd-Elnabi and H.A.H. Ibrahim, 2009. Bio-Control of *Vibrio fluvialis* in Aquaculture by Mangrove (*Avicennia marina*) Seeds Extracts. Research Journal of Microbiology, 4(1): 38-48.
22. Choudhury, S., A. Sree, S.C. Mukherjee, P. Pattnaik, M. Bapuji, 2005. *In vitro* antibacterial activity of extracts of selected marine algae and mangroves against fish pathogens. Asian Fishery Science, 18: 285-294.
23. Mishra, P.V. and A. Sree, 2007. Antibacterial activity and GC-MS analysis of the Extract of Leaves of Finlaysonia obovata (A mangrove plant). Asian journal of Plant Sciences, 6(1): 168-172.
24. Cyrus, W.G., G.W. Daniel, M.O. Nanyingi, F.K. Njonge and J.M. Mbaria, 2008. Antibacterial and cytotoxic activity of Kenyan medicinal plants, Memórias do Instituto Oswaldo Cruz, 103(7): 650-652.
25. Haque, M.E., M.N. Islam, M.H. Rahman and A.U. Mohamad, 2007. Antimicrobial and cytotoxic activities of the crude extracts and isolated compounds of *Xylocarpus mollucensis,* Dhaka University Journal of Pharmaceutical Science, 6(2): 109-112.
26. Bandaranayake, W.M., 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management, 10: 421-452.
27. Han, L., X. Huang, I. Sattler, H.M. Dahse, H. Fu, W. Lin and S. Grable, 2004. New Diterpenoids from the Marine Mangrove *Bruguiera gymnorrhiza.* Journal of Natural Product, 67(9): 1620-1623.

28. Hewage, C.M., B.M.R. Bandara, V. Karunaratne, G.P. Wannigama, M.R.M. Pinto and D.S.A. Wijesundara, 1998. Antibacterial activity of some medicinal plants of Sri Lanka. Journal of Natural Science Council of Sri Lanka, 26: 27-34.
29. Yoshida, T. and H. Ito, 2000. Current Topics in Phytochemistry," Vol. 4, Eds. By Asakawa, Y., O.R. Gottlieb, K. Hostettmann, G.H.N. Towers, H. Wagner, P.G, Waterman Research Trends, India, pp. 135-145.
30. Fukami, H. and M. Nakajima, 1971. Naturally Occurring Insecticides," Eds. By Jacobson, M., D.G. Crosby, Dekker, New York.
31. Bhargava, U.C. and B.A. Westfall, 1968. Antitumor activity of *Juglans nigra* (Black Walnut) extractives. Journal of Pharmaceutical Sciences, 57: 1674-1677.
32. Mars, W.B., M.C. Donascimento, J.R. Do Valle and J.A. Aragao, 1973. Ichthyotoxic activity of plants of the genus Derris and compounds isolated there from. Ciencia Cultura, 25: 647-648.
33. Teixeira, J.R.M., A.J. Lapa, C. Souccar and J.R. Valle, 1984. Timbos: ichthyotoxic plants used by Brazilian Indians. Journal of Ethanopharmocolgy, 10: 311-318.
34. Gomez, E.D., A.A. De La Cruz, B.S. Joshi, V. Chittawong and D.H. Miles, 1989. Toxicants from mangrove plants, V. Isolation of piscicide 2-hydroxy-5-methoxy-3-undecyl-1,4- benzoquinone (5–0-methylembelin) from *Aegiceras corniculatum*. Journal of Natural Product, 52: 649-651.
35. Ufodike, E.B.C. and E. Omoregie, 1994. Acute toxicity of water extracts of barks of *Balanites aegyptiaca* and *Kigelia africana* to *Oreochromis niloticus* (L). Aquaculture and Fisheries Management, 25: 873-879.
36. Madhu, K. and R. Madhu, 1997. Biotoxicity of mangroves on fingerlings of *Liza macrolepis* (Smith). Journal of the Andaman Science Association, 13: 59-65.
37. Miki, W., K. Kon-ya and S. Mizobuchi, 1996. Biofouling and marine biotechnology: New antifoulants from marine invertebrates. Journal of Marine Biotechnology, 4: 117-120.
38. Devi, P., J. Vennam, C.G. Naik, P.S. Parameshwaran, T.V. Raveendran and K.S. Yeshwant, 1998. Antifouling activity of Indian marine invertebrates against the green mussel *Perna viridis* L. Journal of Marine Biotechnology, 6: 229-232.
39. Chen, J.D., D.Q. Feng, Z.W. Yang, Z.C. Wang, Y. Qiu and Y.M. Lin, 2008. Antifouling Metabolites from the Mangrove Plant *Ceriops tagal.* Molecules, 13: 212-219.
40. McGaw, L.J., A.K. Jäger, J. Van Staden, 2002. Isolation of antibacterial fatty acids from *Schotia brachypetala*. Fitoterapia, 73: 431-433.
41. Venkatesalu, V., P. Sundaramoorthy, M. Anantharaj, M. Gopalakrishnan and M. Chandrasekaran, 2004. Studies on the fatty acid composition of marine algae of Rameswaram coast. Seaweed Research Utilization, 26: 83-86.
42. Seidel, V. and P.W. Taylor, 2004. *In vitro* activity of extracts and constituents of *Pelagonium* against rapidly growing mycobacteria. International Journal of Antimicrobial Agent, 23: 613-619.
43. Agoramoorthy, G., M. Chandrasekaran, V. Venkatesalu and M.J. Hsu, 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Brazilian Journal of Microbiology, 38: 739-742.